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TECHNICAL MANUSCRIPT 439

INDUCTION OF INTERFERON BY COXIELLA BURNETII IN CELL CULTURES

Nicholas Hahon
Edmund H. Kozikowski

MARCH 1968

DEPARTMENT OF THE ARMY
Fort Detrick
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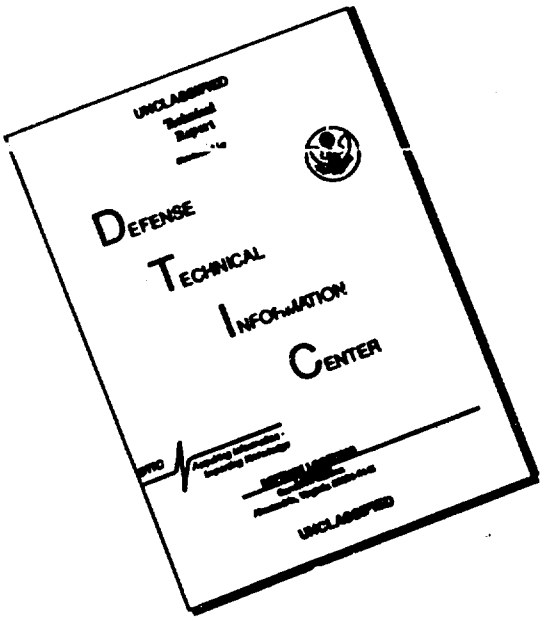

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DEPARTMENT OF THE ARMY
Fort Detrick
Frederick, Maryland 21701

TECHNICAL MANUSCRIPT 439

INDUCTION OF INTERFERON BY *COXIELLA BURNETII* IN CELL CULTURES

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AEROBIOLOGY AND EVALUATION LABORATORY

Project 1B522301A080

March 1968

ABSTRACT

The selective induction of interferon in McCoy and L cell cultures by Coxiella burnetii is described. Test preparations, assayed in homologous cell cultures, showed that significant amounts of interferon were produced from McCoy cells in the absence of rickettsial multiplication. In contrast, no measurable amounts of interferon were produced from L cells that supported rickettsial multiplication. Rickettsial interferon possessed biological and physical properties similar to those ascribed to viral interferon.

INDUCTION OF INTERFERON BY COXIELLA BURNETII IN CELL CULTURES

The production of interferon or interferon-like inhibitors has been induced by practically every major viral group and by nonviral substances that include bacteria, bacterial endotoxins, statolon, phytohemagglutinins, and the nucleic acids and their derivatives.[1] Although in vivo induction of an interferon-like inhibitor by Coxiella burnetii and Rickettsia prowazekii has been demonstrated,[2] the only rickettsial agent reported to induce the production of interferon in cell culture is Rickettsia tsutsugamushi.[3] The present communication describes the selective induction of interferon in cell cultures by another rickettsia, C. burnetii.

A partially purified suspension of the AD (California bovine) strain of C. burnetii containing 1 x 10^6 cell-infecting units/ml, prepared and assayed in accord with procedures described previously,[4] was employed for induction of interferon in L strain mouse fibroblasts and McCoy (human origin) cell cultures. Nutrient medium for both cell lines consisted of medium 199 with 0.5% (w/v) lactalbumin hydrolyzate and 10% (v/v) calf serum. Cells were maintained in medium 199 with 5% (v/v) calf serum. For interferon production, 1:4 dilution of rickettsial suspension was introduced in 4-ml volume into 75 cm^2 tissue culture flasks containing cell monolayers and incubated at 35 C for 3 hours. Residual inoculum was then removed, the cell monolayers were washed, and 5 ml of maintenance medium were added. After incubation at 35 C for 24 hours, supernatant fluids were removed, centrifuged at 105,000 x g for 2 hours, and dialyzed against HCl-KCl buffer, pH 2.0, and Earle's BSS, pH 7.1, each at 4 C for 24 hours.

Preparations were assayed for interferon activity by a procedure based on the 50% reduction of yellow fever virus fluorescent cell counts.* Interferon assays were usually carried out in quadruplicate. Briefly, the procedure consisted of introducing appropriate dilutions of test preparations or control medium in 0.5-ml volume onto cover slip cell monolayers and incubating at 35 C for 24 hours. Approximately 7 x 10^3 cell-infecting units of the Asibi strain of yellow fever virus, the challenge agent, were then introduced in 0.2-ml volume onto each cover slip cell monolayer. Inoculum was centrifuged at 29,000 x g for 15 minutes to promote efficient and rapid attachment of virus to cells. A description of the fluorescent cell-counting assay procedure of yellow fever virus has been reported in detail elsewhere.[5] The reciprocal of the interferon dilution that reduced the number of fluorescent cells to 50% of the controls served as the measure of potency of interferon preparations. The 50% reduction value was derived by plotting probit transformations of reduction percentages against corresponding interferon dilutions.

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* Kozikowski, E.H., and N. Hahon, unpublished data.

Results in Table 1 show that the yield of interferon produced by C. burnetii was significantly larger in McCoy than in L cells. This was evident only when test preparations were assayed in homologous cell cultures. The relative insensitivity of L cells to interferon produced in McCoy cells demonstrates the cell species specificity of interferon. Although C. burnetii has been shown to grow in L cell cultures,[4,6] multiplication of this rickettsia in McCoy cells could not be demonstrated either by inoculation of chick embryonated eggs or by fluorescent-antibody procedures.* That C. burnetii multiplies in L cells but does not induce measurable amounts of interferon and, conversely, produces good yields of interferon but does not grow in McCoy cells is similar to findings reported for the interferon induction activities of Newcastle disease virus.[7] The possibility of interferon induction in L cells by inactivated C. burnetii, e.g., priming phenomenon,[8] is not excluded by this preliminary study. In contrast to our results that induction of interferon by C. burnetii occurs in the absence of rickettsial multiplication, interferon induction in primary chick cells by R. tsutsugamushi was dependent on rickettsial growth.[3] From our cursory observations, it appears that the complex relationships reported between viral inducers and cells[9] are also in evidence in the interaction between rickettsial inducers and cell cultures with each playing an important role as a determinant of interferon production.

TABLE 1. INDUCTION OF INTERFERON BY COXIELLA BURNETII AND ITS ASSAY IN HOMOLOGOUS AND HETEROLOGOUS CELL LINES

Cell Line for Interferon Induction	Cell Line for Interferon Assay	
	McCoy	L
McCoy	200[a]/	<10
L	<10	<10

a. Reciprocal dilution of preparations in 0.5-ml volume that reduced the number of yellow fever virus fluorescent cells by 50% of controls.

That the preparation induced by C. burnetii in McCoy cells possessed biological and physical properties similar to those ascribed to viral interferon was established from determinations of cell species specificity, inhibition of viral multiplication, non-sedimentation at 100,000 x g, nondialyzability, resistance to pH 2.0, sensitivity to trypsin, and stability at 60 C for 1 hour but not at 80 C for 1 hour.

* Hahon, N., unpublished data.

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14. Key Words
*Interferon
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